

# Study of Genetic Variation of Captive Asiatic Golden Cat (*Pardofelis temminckii*) in Thailand Using Domestic Cat (*Felis catus*) Microsatellite Markers

Suwimon Phandee<sup>1,2,3,6</sup> Janjira Phavaphutanon<sup>4</sup> Kaikanoke Sirinarumitr<sup>4</sup>

Sudtisa Laopiem<sup>5</sup> Theerapol Sirinarumitr<sup>3\*</sup>

## Abstract

Domestic cat (*Felis catus*) microsatellite markers were used to determine genetic diversity in 17 captive Asiatic golden cats (*Pardofelis temminckii*, syn. *Catopuma temminckii*). Allele numbers, genotype numbers, genetic diversity, heterozygosity, polymorphism information content (PIC) and inbreeding coefficient (*f*) were observed in each marker. The average values of genotype number, allele number, expected heterozygosity, observed heterozygosity, polymorphism information content (PIC) and inbreeding coefficient (*f*) were 6.12, 4.71, 0.70, 0.29, 0.65 and 0.62, respectively. These results revealed a reduction in the genetic diversity as shown by the lower value for the observed heterozygosity compared to the expected heterozygosity and the high value of the inbreeding coefficient. In conclusion, domestic cat microsatellite markers can be used to study captive Asiatic golden cat genetic diversity and to plan a breeding program for captive Asiatic golden cat for conservation.

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**Keywords:** Asiatic golden cat, Felidae, genetic diversity, microsatellite markers

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<sup>1</sup>Center for Agricultural Biotechnology, Kasetsart University, Kamphaeng Saen Campus, Nakhon Pathom 73140, Thailand

<sup>2</sup>Center of Excellence on Agricultural Biotechnology, Science and Technology Postgraduate Education and Research Development Office, Commission on Higher Education, Ministry of Education, Thailand (AG-BIO/PERDO-CHE)

<sup>3</sup>Department of Pathology, Faculty of Veterinary Medicine, Kasetsart University, Bangkok 10900

<sup>4</sup>Department of Companion Animals Clinical Sciences, Faculty of Veterinary Medicine, Kasetsart University, Kamphaeng Saen Campus, Nakhon Pathom 73140, Thailand

<sup>5</sup>Department of Farm Resources and Production Medicine, Faculty of Veterinary Medicine, Kasetsart University, Kamphaeng Saen Campus, Nakhon Pathom 73140, Thailand

<sup>6</sup>Center for Advanced Studies in Agriculture and Food, Kasetsart University Institute for Advanced Studies, Kasetsart University, Bangkok 10900, Thailand (CASAF, NRU-KU, Thailand)

\*Correspondence: foettps@yahoo.com

## Introduction

The Asiatic golden cat (*Pardofelis temminckii*, syn. *Capopuma temminckii*), a medium-sized wild cat, lives throughout South Asian and Southeast Asian countries including Nepal, Tibet, Bhutan, Burma, Cambodia, Thailand, Vietnam, China, Bangladesh, India, Lao, Malaysia and Sumatra (Olsen, 2012). The Asiatic golden cat was classified in the species listed in Appendix I of CITES (Conservation on International Trade in Endangered Species of Wild Fauna and Flora), which includes species threatened with extinction and so trade in these species is only permitted under exceptional circumstances (Randi et al., 2002). Moreover, it was categorized as Vulnerable (VU) on the IUCN (International Union for the Conservation of Nature) Red List 2002, which means it is at high risk of endangerment in the wild (Traylor-Holzer et al., 2005; Srisamoot et al., 2007). In 2010, the IUCN classified this species as Near Threatened (NT), meaning likely to become endangered in the near future and it is still listed in Appendix I of CITES (Olsen, 2012). This species has been threatened continually by deforestation, which has reduced the habitat for wild animals and caused population fragmentation, which leads to the loss of their genetic diversity. Because this species is difficult to breed in captivity, they are at high risk of extinction. However, there are few data about their habits, social structure and breeding behavior (Olsen, 2012). It is important to know the genetic relationship and to use the data for breeding management for conservation in such species and prevention of a reduction in their genetic diversity as a result of inbreeding.

In conservation genetics, it is of principal importance to have a breeding program to reduce inbreeding in captive animals. Inbreeding causes a reduction in genetic variability, fertility and survival rate, which lead to an increase in the extinction risk (Arif and Khan, 2009). DNA molecular methods are tools that can be used to solve the loss of genetic heterogeneity in endangered wild animals. Microsatellites are available for the detection of genetic diversity and are present in many thousands of loci. These microsatellites are composed of small DNA fragments that contain repetitive elements displaying tandem repeats of the 1-6 base pair motifs. Variations in the number of repeat motifs result in loci of high polymorphic information content (PIC) and microsatellites are co-dominant markers with bi-allelic or multi-allelic performance in an individual or a population, respectively. Microsatellites have been used as molecular markers in studying conservation genetics, investigating forensic identification, profiling DNA fingerprints and analyzing parentage (Menotti-Raymond and O'Brien, 1995; Culver et al., 2001; O'Brien et al., 2002; Arif and Khan, 2009).

Menotti-Raymond et al. (1995) demonstrated 10 domestic cat microsatellite markers (FCA008, FCA023, FCA035, FCA043, FCA045, FCA077, FCA078, FCA090, FCA096 and FCA126) that could amplify DNA samples from lion, cheetah, puma, Asian leopard cat and Geoffrey's cat. Hille et al. (2000) studied the genetic individualization of the European wildcat (*Felis silvestris*) and domestic cat using a panel of eight feline

microsatellite markers (F115, FCA031, FCA035, FCA096, FCA105, FCA124, FCA126 and FCA132). Eizirk et al. (2001) investigated genetic diversity in jaguar (*Panthera onca*) using 29 domestic cat microsatellite markers. Moreno et al. (2006) used four domestic cat microsatellite loci (FCA008, FCA045, FCA077 and FCA096) to investigate genetic variability in specimens of Eyra cat (*Herpailurus yagouaroundi*), cougar (*Puma concolor*) and jaguar (*Panthera onca*). Moreover, Grisolia et al. (2007) demonstrated that the same four pairs of domestic cat microsatellite markers could amplify products from ocelot (*Leopardus pardalis*), margay (*Leopardus wiedii*) and oncilla (*Leopardus tigrinus*) because the domestic cat and other species of the felid family, Felidae, had evolved within 10 million years and the genomes of the Felidae family species were almost identical to the domestic cat, with 15 of 19 domestic cat chromosomes constant among all the other Felidae species. Thus, the genomic tools developed for domestic cat can be applied to the conservation genetics of exotic felids (O'Brien et al., 2002; Menotti-Raymond et al., 2003). Therefore, the objective of this study was to determine whether domestic cat microsatellite markers can be used to study genetic diversity and to plan a breeding program for captive Asiatic golden cats for conservation.

## Materials and Methods

**Animals and sample collection:** One milliliter of EDTA blood sample was collected from each of 17 Asiatic golden cats (*Pardofelis temminckii*) from Songkhla Zoo (n=7), Khaohekheaw Zoo (n=2), Chiangmai Zoo (n=4) and KhaoPratubchang Wildlife Breeding Center (n=4). The EDTA blood samples were kept at -20°C until used.

**DNA extraction:** The EDTA blood samples were subjected to DNA extraction using the phenol-chloroform extraction method (Sambrook et al., 1989). Concentration and purity of the extracted DNA was measured using a spectrophotometer (SmartSpec™ Plus Spectrophotometer, BIO-RAD, USA).

**DNA amplification:** Seventeen microsatellite markers for domestic cat (Menotti-Raymond and O'Brien, 1995; Menotti-Raymond et al., 1999; Menotti-Raymond et al., 2005) were used in this study (Table 1). Twenty microliters of the PCR mixture (Invitrogen®, Brazil) was composed of 50 ng of DNA template, 2 µl of PCR buffer (10xbuffer), 1 µl of 50 mM MgCl<sub>2</sub>, 0.4 µl of 10 mM dNTPs, 0.1 µl of each forward and reverse primers (100 µM), 0.1 µl of Taq DNA polymerase and DNase-free water. The PCR conditions were modified from those described earlier (Menotti-Raymond et al., 1999). After an initial denaturation at 93°C for 3 min; the amplification was performed by 10 cycles of 94°C for 15s, 55°C for 15s, 72°C for 30s; followed by 20 cycles of 89°C for 15s, 55°C for 15s, and 72°C for 30s and a final extension at 72°C for 30 min. The PCR products were electrophoresed using 1.5% agarose gel (ADVANCE, Japan) and visualized under ultraviolet illumination. DNA fragment analysis was performed using automated capillary electrophoresis (QIAGEN®, Germany).

**Statistical analysis:** The size of alleles for loci was analyzed using PowerMarker Version 3.25. Allele numbers, genotype numbers, genetic diversity, heterozygosity, polymorphism informative content (PIC) and inbreeding coefficient ( $f$ ) were calculated for each marker. The numbers of alleles and genotypes, which correlate, were used to describe genetic diversity. The polymorphism informative content (PIC) values were used to determine potential

usefulness of markers for each locus. Genetic diversity, often referred to as expected heterozygosity, is defined as the probability which the randomly chosen alleles from the population are different. Heterozygosity refers to the observed heterozygosity. The inbreeding coefficient ( $f$ ) is a relative measure, in that there will be a certain level of homozygosity within the population and it estimates the increase from that initial level as a result of recent inbreeding.

**Table 1** Seventeen microsatellite markers for domestic cat used in this study

Microsatellite loci	Chromosomal assignment	Primer sequence
F42	A1	5'CCCACGTGGACTAATCAAAT3' 3'CACTGCACA AAT TAAGAGGC5'
F53	A1	5'GTTGGGAGTAGAGATCACCT3' 3'GAAAAAGACTCCTGCTTGCA5'
F124	E1	5'TGCTGGTATGAAGCCTACT3' 3'ATTGCCTCAACTACCTAGGC5'
F164	C2	5'CTATATGACAACCTGAGAACT3' 3'AGATGATACAGGTAGAGGTC5'
FCA008	A1	5'ACTGTAAATTTCTGAGCTGGCC3' 3'TGACAGACTGTTCTGGGTATGG5'
FCA035	D2	5'CTTGCCTCTGAAAAATGTAAAATG3' 3'AAACGTAGGTGGGT TAGTGG5'
FCA045	A1	5'TGAAGAAAAGAATCAGGCTGTG3' 3'GTATGAGCATCTCTGTGTTCGTG5'
FCA077	C2	5'GGCACCTATAACTACCAGTGTGA3' 3'ATCTCTGGGGAAATAAATTTTGG5'
FCA096	A2	5'CACGCCAAACTCTATGCTGA3' 3'CAATGTGCCGTCCAAGAAC5'
FCA105	A2	5'TTGACCTCATACCTTCTTTGG3' 3'TGGGAGAATAAATTTGCAAAGC5'
FCA124	A2	5'CCATTCCTCCCTGCTCTGTA3' 3'GCCTCAAGCCTCATTTGCTAC5'
FCA126	B1	5'GCCCCTGATACCCTGAATG3' 3'CTATCCTTGCTGGCTGAAGG5'
FCA391	B3	5'GCCTTCTAACTTCCTGCAGA3' 3'TTTAGGTAGCCATTTTCATCA5'
FCA665	A2	5'AACCTGCCTGAGCCAGTG3' 3'TCGGAGAAA ATT TCCAGGGGCT5'
FCA726	A2	5'GCACAGAGGATTCCCCATAA3' 3'GCCCCGTGTTGCTGTGTACT5'
FCA739	C1	5'GTGCTGTATTTGTATCTGTATCTGT3' 3'AAAGGGAAGTGACCACTGGA5'
FCA747	D4	5'GCCTCTTTGGCAACCATTAG3' 3'TCTTGGAATTACTCCTGGTAAACA5'

## Results

All 17 microsatellite markers for domestic cat could amplify the DNA samples from the Asiatic golden cats. The size of these PCR products for each locus is shown in Table 2. The allele sizes of F42, F164, FCA096, FCA105 and FCA665 in the Asiatic golden cat were larger than those of domestic cat and the allele sizes of F53, FCA077 and FCA739 in the Asiatic golden cat were smaller than those of domestic cat. These loci showed different alleles with no overlapping size ranges for the Asiatic golden cat and domestic cat.

The averages of the genotype number, allele number, genetic diversity (expected heterozygosity), heterozygosity (observed heterozygosity), polymorphism information content (PIC) and inbreeding coefficient ( $f$ ) were 6.12, 4.71, 0.70, 0.29, 0.65 and 0.62, respectively (Table 3). The results in each locus are shown in Table 3. Five microsatellite markers used in this study (FCA008, FCA035, FCA077, FCA096 and FCA124) had null values for the heterozygosity. These results revealed reduction in genetic diversity due to the high value of the inbreeding coefficient and the decreased observed heterozygosity value.

**Table 2** Characterization of PCR product sizes in Asiatic golden cat

Microsatellite loci	PCR product size range (bp)	
	Domestic cat <sup>1</sup>	Asiatic golden cat <sup>2</sup>
F42	205-231	241-297
F53	288-344	160-180
F124	110-134	128-142
F164	146-166	216-248
FCA008	122-148	146-158
FCA035	136-150	132-136
FCA045	146-160	148-172
FCA077	143-155	127-133
FCA096	184-224	230-248
FCA105	189-197	201-221
FCA124	208-248	236-264
FCA126	139-145	137-161
FCA391	237-273	241-265
FCA665	234-244	248-256
FCA726	229-245	243-251
FCA739	246-278	182-230
FCA747	134-144	138-158

<sup>1</sup>From Menotti-Raymond and O'Brien, 1995; Menotti-Raymond et al., 1999; Menotti-Raymond et al., 2005

<sup>2</sup>This study

## Discussion

In this study, all domestic cat microsatellite markers could amplify the DNA from all 17 Asiatic golden cats. This result implied the success in interspecies microsatellites amplification using domestic cat microsatellite markers, which indicates the conservation of the flanking primer sequence between species (Grisolia et al., 2007).

The PIC values of each microsatellite locus for the Asiatic golden cats were 0.46-0.86 and the average PIC was 0.65. A polymorphism informative content (PIC) value higher than 0.7 indicates high polymorphism and can be used to characterize individuals, whereas a value between 0.44 and 0.7 is considered to be moderately informative (Hildebrand et al., 1992; Moreno et al., 2006). Thus, the results of PIC in this study indicated a medium to high efficiency rate of the domestic cat microsatellite markers used in this study for characterization of the genetic variability of Asiatic golden cat.

The numbers of alleles and genotypes described the genetic diversity and both correlated. A high allele number for microsatellite loci can be related to multiallelic performance. High heterozygosity of loci indicates a variety of genotypes. In this study, the average genotype number (6.12) and allele number (4.71) were moderate values. Grisolia et al. (2007) studied genetic diversity in 148 captive *Leopardus* spp. using four domestic cat markers which were shown to have an average number of alleles of 12.5, 11.5 and 11

in *Leopardus pardalis*, *Leopardus tigrinus* and *Leopardus wiedii*, respectively. These results implied high genetic diversity among the species studied. Menotti-Raymond et al. (1995) reported that the heterozygosity values of FCA008, FCA035, FCA077 and FCA096 in domestic cat were 0.89, 0.60, 0.63 and 0.85, respectively; in cheetah were 0.84, 0.60, 0.00 and 0.74, respectively; in puma were 0.49, 0.50, 0.62 and 0.82, respectively; and in lion were 0.73, 0.79, 0.76 and 0.74, respectively. Moreover, they studied the evolutionary conservation of 10 microsatellite loci in four species of felids and demonstrated high polymorphism in puma, lion, cheetah and domestic cat. However, the cheetah showed the lowest level of polymorphism for these loci among the felid species. There was a wide range in the heterozygosity level for 10 microsatellite loci from 0 to 0.842 but the heterozygosity of FCA043, FCA077 and FCA090 was 0.00, which meant that the genetic diversity of these loci was reduced to monomorphism at the time of the bottleneck. As shown in this study, five domestic cat microsatellite loci (FCA008, FCA035, FCA077, FCA096 and FCA124) had null values for the observed heterozygosity, which indicated a homozygous genotype at these loci. The observed heterozygosity values ranged from 0 to 0.93 and the average of the observed heterozygosity was 0.29. Moreover, the expected heterozygosity ranged from 0.52 to 0.88 and the average expected heterozygosity was 0.70. The observed heterozygosity level was less than the expected heterozygosity, which meant a

reduction in the genetic diversity in these captive Asiatic golden cats.

The allele sizes of F42, F164, FCA096, FCA105 and FCA665 in the Asiatic golden cat were larger than those seen in domestic cat and the allele sizes of F53, FCA077 and FCA739 in the Asiatic golden cat were smaller than those seen in domestic cat. These results suggest differences in the mutation rate for individual microsatellite loci. Differences among alleles were likely due to inter-specific biological diversity resulting from the mutation of microsatellite markers (Grisolia et al., 2007). These allele sizes showing different alleles with no overlapping size ranges for the Asiatic golden cats and domestic cats indicated that these alleles were exclusive alleles and implied species identification between the Asiatic golden cat and domestic cats. Unfortunately, the sample size in this study was too small to support this conclusion. The average inbreeding coefficient was 0.62, which tended toward a high value (close to 1). Inbreeding causes an increase

in homozygous genotype frequencies and a decrease in heterozygosity (Randi et al., 2002); homozygous genotypes were shown in 5 out of the 17 microsatellite loci in this study. This study revealed the reduction in the genetic diversity in captive Asiatic golden cat through the high value of the inbreeding coefficient and the lower observed heterozygosity value than the expected heterozygosity. Moreover, this study demonstrated that the situation of captive Asiatic golden cat in Thailand is critical and this species is vulnerable to loss of genetic diversity. Authorities should implement a mating management plan to solve the inbreeding problem and increase the genetic diversity in the Asiatic golden cat. In conclusion, the domestic cat microsatellite markers used in this study can provide genetic diversity data for monitoring the genetic variability of species and can assist in planning a reproductive program for the captive Asiatic golden cats in Thailand.

**Table 3** Microsatellite loci, sample size, genotype number, allele number, genetic diversity, heterozygosity, polymorphism information content (PIC) and inbreeding coefficient (*f*) in Asiatic golden cat

Microsatellite loci	Sample size	Genotype no	Allele no	Genetic diversity (Expected heterozygosity)	Heterozygosity (Observed heterozygosity)	PIC	<i>f</i>
F042	17.00	12.00	9.00	0.88	0.93	0.86 <sup>a</sup>	-0.03
F124	17.00	8.00	6.00	0.81	0.30	0.78 <sup>a</sup>	0.66
FCA126	17.00	9.00	6.00	0.81	0.58	0.78 <sup>a</sup>	0.32
F164	17.00	6.00	6.00	0.78	0.78	0.75 <sup>a</sup>	0.07
FCA391	17.00	7.00	5.00	0.77	0.38	0.73 <sup>a</sup>	0.53
FCA105	17.00	7.00	6.00	0.75	0.38	0.72 <sup>a</sup>	0.52
FCA008	17.00	5.00	5.00	0.71	0.00	0.67 <sup>b</sup>	1.00
FCA124	17.00	4.00	4.00	0.72	0.00	0.67 <sup>b</sup>	1.00
F53	17.00	7.00	5.00	0.70	0.19	0.65 <sup>b</sup>	0.75
FCA739	17.00	7.00	4.00	0.70	0.18	0.65 <sup>b</sup>	0.76
FCA045	17.00	7.00	4.00	0.68	0.71	0.61 <sup>b</sup>	-0.02
FCA096	17.00	4.00	4.00	0.66	0.00	0.61 <sup>b</sup>	1.00
FCA747	17.00	7.00	4.00	0.65	0.29	0.60 <sup>b</sup>	0.57
FCA035	17.00	3.00	3.00	0.62	0.00	0.55 <sup>b</sup>	1.00
FCA665	17.00	3.00	3.00	0.60	0.00	0.52 <sup>b</sup>	1.00
FCA726	17.00	5.00	3.00	0.58	0.12	0.51 <sup>b</sup>	0.81
FCA077	17.00	3.00	3.00	0.52	0.00	0.46 <sup>b</sup>	1.00
<b>Mean</b>	<b>17.00</b>	<b>6.12</b>	<b>4.71</b>	<b>0.70</b>	<b>0.29</b>	<b>0.65</b>	<b>0.62</b>

<sup>a</sup>PIC value considered to be highly informative

<sup>b</sup>PIC value considered to be moderately informative

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## บทคัดย่อ

### การศึกษาความหลากหลายทางพันธุกรรมโดยใช้เครื่องหมายพันธุกรรม

#### ไมโครแซทเทลไลท์แมวบ้านในเสือไฟ

สุวิมล พันธุ์ดี<sup>1,2,3,6</sup> จันทร์จิรา ภาณุตานนท์<sup>4</sup> เกษกนก ศิริณฤมิตร<sup>4</sup> สุธริษา เหล่าเปี่ยม<sup>5</sup> อีระพล ศิริณฤมิตร<sup>3\*</sup>

การศึกษาความหลากหลายทางพันธุกรรมของเสือไฟ (*Pardofelis temminckii*) 17 ตัวที่ถูกเลี้ยงอยู่ในสวนสัตว์โดยใช้เครื่องหมายไมโครแซทเทลไลท์แมวบ้าน (domestic cat microsatellite markers) การศึกษาพบว่า ค่าเฉลี่ยในแต่ละตำแหน่งของเครื่องหมายไมโครแซทเทลไลท์ ได้แก่ จำนวนจีโนไทป์ (genotype numbers) จำนวนอัลลีล (allele numbers) ค่าการคาดหมายเฮเทอโรซัยกอส (expected heterozygosity) ค่าการสังเกตเฮเทอโรซัยกอส (observed heterozygosity) ค่า polymorphism information content (PIC) และค่า inbreeding coefficient (*f*) คือ 6.12, 4.71, 0.70, 0.29, 0.65 และ 0.62 ตามลำดับ ซึ่งค่าการสังเกตเฮเทอโรซัยกอสต่ำกว่าค่าการคาดหมายเฮเทอโรซัยกอสแสดงให้เห็นว่าเสือไฟในการศึกษานี้มีความหลากหลายทางพันธุกรรมที่ต่ำ หรือมีการผสมพันธุ์ในเครือญาติ (inbreeding) สอดคล้องกับค่า inbreeding coefficient (*f*) ที่ค่อนข้างสูง จากผลการศึกษานี้สรุปได้ว่า สามารถใช้เครื่องหมายไมโครแซทเทลไลท์แมวบ้านกับเสือไฟได้และนำเทคนิคนี้มาประยุกต์ใช้ในการวางแผนโปรแกรมในการผสมพันธุ์เสือไฟเพื่อลดการผสมพันธุ์กันระหว่างเครือญาติ

**คำสำคัญ:** เสือไฟ สัตว์ตระกูลแมว ความหลากหลายทางพันธุกรรม เครื่องหมายไมโครแซทเทลไลท์

<sup>1</sup>ศูนย์เทคโนโลยีชีวภาพเกษตร มหาวิทยาลัยเกษตรศาสตร์ วิทยาเขตกำแพงแสน จ.นครปฐม 73140

<sup>2</sup>ศูนย์ความเป็นเลิศด้านเทคโนโลยีชีวภาพเกษตร สำนักพัฒนาบัณฑิตศึกษาและวิจัยด้านวิทยาศาสตร์และเทคโนโลยี สำนักงานคณะกรรมการการอุดมศึกษา กรุงเทพฯ 10900

<sup>3</sup>ภาควิชาพยาธิวิทยา คณะสัตวแพทยศาสตร์ มหาวิทยาลัยเกษตรศาสตร์ กรุงเทพฯ 10900

<sup>4</sup>ภาควิชาเวชศาสตร์คลินิกสัตว์เลี้ยง คณะสัตวแพทยศาสตร์ มหาวิทยาลัยเกษตรศาสตร์ วิทยาเขตกำแพงแสน จ.นครปฐม 73140

<sup>5</sup>ภาควิชาเวชศาสตร์และทรัพยากรการผลิตสัตว์ คณะสัตวแพทยศาสตร์ มหาวิทยาลัยเกษตรศาสตร์ วิทยาเขตกำแพงแสน จ.นครปฐม 73140

<sup>6</sup>ศูนย์วิทยาการขั้นสูงเพื่อเกษตรและอาหาร มหาวิทยาลัยเกษตรศาสตร์ กรุงเทพฯ 10900

\*ผู้รับผิดชอบบทความ E-mail: fvettps@yahoo.com